Research Article

# Neuroprotective effects of the novel ethylthiadiazole derivatives (LHT 4–15) in male rats

**Mikhail A. Martynov*, Roman F. Cherevatenko, Olga V. Martynova, Alena S. Timokhina, Natalia V. Ekimova, Natalia I. Nesterova, Sergey V. Povetkin, Natalia N. Bystrova, Anna A. Peresypkina, Natalia D. Bunatyan**

## ABSTRACT

**Introduction:** The study was to investigate the ethylthiadiazole derivative with the (code LHT 4–15) for the presence of neuroprotective effects. **Materials and Methods:** The study was performed on male rats of the Wistar line. The animals were simulated with total cerebral ischemia with preliminary administration of LHT 4–15 compounds in doses of 25 and 50 mg/kg for 60 min. The degree of neurological deficiency and the behavioral status of the animal was studied. In the experiment, five groups of rats ($n$ = 10) were isolated: (1) intact, (2) pseudo-operated, (3) with total cerebral ischemia, (4) with total cerebral ischemia and 60 min LHT 4–15 in a dose of 25 mg/kg, and (5) with total cerebral ischemia and a preliminary dose of 50 mg/kg in 60 min. In the work, a pathology model was used in which a temporary occlusion of two common carotid arteries was performed for 4 min, with preliminary coagulation of two vertebral arteries. The protocol of the study included the following stages: Modeling of cerebral ischemia; evaluation of the electroencephalogram level of the animal; and assessment of behavioral status (2 days) and neurological deficit at 1, 3, 7, and 14 days after modeling pathology. The blood of rodents was investigated in the contents of s100b and neuron-specific enolase (NSE). **Results:** The introduction of LHT 4–15 at a dose of 25 and 50 mg/kg limited the development of neurologic deficits ($P < 0.05$). After 1 day, the average neurological ball in the group with the administration of LHT 4–15 at a dose of 25 mg/kg was 1.8 ± 0.11, and when 50 mg/kg was administered 0.7 ± 0.09. In the behavioral test "elevated cross-shaped labyrinth" of a group of rats with cerebral ischemia and preliminary administration of LHT 4–15 at a dose of 25 mg/kg and 50 mg/kg, they showed themselves more actively compared to the control group. This manifested itself in an increase in horizontal and vertical activity, which was reflected in more racks and stilts. The estimated research behavior declined, but insignificantly ($P < 0.05$). When assessing the motor activity of animals in the actinometry test with infrared (IR) monitoring of the IR Actimeter activity, a group of rats with the administration of LHT 4–15 at a dose of 25 mg/kg showed an activity slightly higher than the group administered with the compound at a dose of 50 mg/kg. Groups of animals with the preliminary administration of LHT 4–15 at doses of 25 and 50 mg/kg were more active, developed a high speed, and passed a longer distance. The rest time in these groups is lower in comparison with the control ($P < 0.05$). When analyzing the level of S100b and NSE in animals of the group with cerebral ischemia, with a preliminary introduction of 2-amino-5-ethyl-1,3,4-thiadiazole glee-cyclizine at a dose of 50 mg/kg there was a decrease in the levels of markers in Virginia even below the level of the control group ($P > 0.05$). Correction of ischemic injury 2-amino-5-ethyl-1,3,4-thiadiazole a glycylglycine showed a statistically significant increase in resistance of neurons in the frontal lobe and CA1 region of the hippocampus to hypoxia and ischemia, a significant decrease in the number of dead neurons and the high activity of reparative processes. **Conclusion:** The data obtained from the neurological deficit and in the behavioral tests of the experimental groups confirm the theory of the presence of ethylthiadiazole derivatives under the LHT code 4–15 neuroprotective properties.

**KEY WORDS:** Cerebral ischemia, Ethylthiadiazole derivatives, Rats

## INTRODUCTION

At present, the number of patients with cerebrovascular diseases is steadily increasing. Increasingly, cerebrovascular accident occurs in able bodied and socially active middle-aged people. In recent years, the study of various aspects of cerebrovascular pathology has significantly intensified, which led to significant progress in the prevention, treatment, and recovery after a stroke, as well as to reduce the mortality from this disease.[1] However, despite the availability of a wide range of modern drugs for stroke pharmacotherapy, the outcome of the disease remains extremely unfavorable – the increasingly cerebrovascular accident remains one of

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod 308015, Russia

***Corresponding author:** Mikhail A. Martynov, Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod 308015, Russia. E-mail: martynova_o@bsu.edu.ru

Received on: 14-07-2019; Revised on: 17-08-2019; Accepted on: 21-09-2019

the leading causes of death, and the majority of patients who have had a stroke permanently remain disabled. Moreover, there is currently no universally recognized neuroprotection program that has proven effective in improving the outcome of the disease. Therefore, the search for new compounds with pronounced cytoprotective[2-5] and specific neuroprotective[6-8] activity becomes is one of the top priorities in pharmacology. The main focus of research development is focused on oxidative stress,[9-12] nitrogen oxide imbalance,[13-16] and intracellular secondary messengers.[17,18]

Ethylthiadiazole derivatives have a wide spectrum of pharmacological activity, including neuroprotective effects.[19,20] Among them, there are a large number of compounds with anti-inflammatory, antimicrobial, anticonvulsant, antihypertensive, antioxidant, and antitumor effect.[21,22] The expressed biological activity of these compounds is due to their chemical structure, in fact, thiadiazoles are heterocyclic compounds based on a ring five-membered heterocycle containing two nitrogen atoms and a sulfur atom.

## MATERIALS AND METHODS

The study was performed on 50 mature male Wistar rats of 5–6-month-old age weighing 180–210 g. The content of animals met all the rules of laboratory practice during pre-clinical studies in the territory of the Russian Federation. The animals were kept in standard conditions that corresponded to sanitary rules (No. 1045-73) approved by the Union of Soviet Socialist Republics Ministry of Health on April 6, 1977 for the arrangement, equipment, and maintenance of experimental biological clinics (vivaria) and GOST R 53434-2009. Vivisection was carried out according to the ethical principles of handling laboratory animals. "The European Convention for the protection of vertebral animals used for experimental and other scientific purposes, CETS No.123".

In the experiment, five groups of rats ($n$ = 10) were isolated: (1) Intact, (2) false-operated, (3) with total cerebral ischemia, (4) with total cerebral ischemia and 60 min LHT 4–15 in a dose of 25 mg/kg, and (5) with total cerebral ischemia and a preliminary dose of 50 mg/kg in 60 min. LHT 4–15 was administered intragastrically through the probe. This compound was synthesized in JSC "All-Russian Center for the study of the safety of biologically active substances" (All-Russian Scientific Center for the Safety of Biologically Active Substances, Russia, Staraya Kupavna).

Animalization of animals in the experiment was performed using zoletil 60 mg/kg and chloral hydrate 150 mg/kg.

All experiments were performed in accordance with the methodological recommendations for preclinical study of drugs in cases of cerebral circulation disorders. In the work, a pathology model was used in which a temporary occlusion of two common carotid arteries was performed for 4 min, with preliminary coagulation of two vertebral arteries. Assessment of the adequacy of occlusion of arteries supplying blood to the brain was performed by recording the electrical activity of the brain of the animal on a Biopac Systems Inc. device MP150 electroencephalogram (EEG100C). The method was carried out with the help of the program AcqKnowledge 4.2. The criterion of a correctly executed technique was a decrease in the amplitude of the EEG.

The protocol of the study included the following stages: Modeling of cerebral ischemia; evaluation of the EEG level of the animal; assessment of behavioral status (2 days); and neurological deficit at 1, 3, 7, and 14 days after modeling pathology.

To evaluate the neurological status of rats, several methods were used: (1) The scale of the McGraw stroke assessment in the modification of I.V. Gannushkina. Within the group of rats with signs of neurologic deficiency were divided into animals with mild, moderate, and severe symptoms of neurologic deficiency. If the animal was present with several signs of a neurological deficit, then the scores were summarized. (2) "Elevated cross-shaped labyrinth" – a behavioral test for studying the activity, emotional state, and level of anxiety of laboratory animals during the experiment. The experiment used a labyrinth installation of Panlab Harvard Apparatus LE 846.3. "Infrared (IR) activity monitor" – IR Actimeter allows performing testing of voluntary motor activity, the number and duration of hitching episodes, stereotyped movements, and exploratory behavior in the "perforated field" model under daylight and nighttime conditions used to assess the orienting research behavior. In the experiment, we used the Panlab Harvard Apparatus LE 8825 installation. The blood of rodents was investigated in the contents of s100b and neuron-specific enolase (NSE).

For all data, descriptive statistics were applied. The obtained data were checked for the normal distribution. Using the Shapiro–Wilk criterion, the type of distribution was chosen. In the case of a normal distribution, the average value of M and the standard error of the mean m was calculated. In cases of abnormal distribution, the median Me and the QR-range were calculated. Intergroup differences were analyzed by parametric (student's $t$-test) or non-parametric (Mann–Whitney test) methods, depending on the type of distribution. Differences were determined at 0.05 significance level. Statistical analysis is performed using the software Statistica 10.0.

## RESULTS AND DISCUSSION

For the control, the data received from animals with total cerebral ischemia were accepted. In assessing the severity of the neurological deficit by McGraw in the modification of I.V. Gannushkina after modeling ischemia, after 1 day, the average ball was high 3.95 ± 0.57, which is due to the presence of symptoms such as lethargy and slowed movements, one-sided half ptosis of the right eye. In 20% of the animals, a death was recorded. Sluggishness and slowness of movements to day 3 after modeling pathologies disappeared. At 3, 7, and 14 days, a semiptosis of the right eye was preserved.

The introduction of LHT 4–15 at a dose of 25 mg/kg limited the development of neurological deficits. After 1 day, the average neurological ball was 1.8 ± 0.11. The neurological deficit manifested itself in sluggishness and slowness, movements, and unilateral half ptosis of the right eye. The percentage of rats with semiptosis was lower than in the group of rats with ischemia. Fixation of 10% of the lethal outcome sluggishness and slowness of movements to day 3 after modeling the pathology disappeared ($P < 0.05$). Compared with the control, the animals of this group were very active even in the 1st day after modeling the pathology.

The introduction of LHT 4–15 at a dose of 50 mg/kg also limited the development of neurological deficits. After 1 day, the average neurological score was 0.7 ± 0.09. The neurological deficit manifested itself in sluggishness and slowness, movements, and unilateral half ptosis of the right eye. Sluggishness and slowness of movements to day 3 after modeling pathologies disappeared. In this group, mortality was not observed in animals ($P < 0.05$) [Table 1].

Thus, the preliminary administration of LHT 4–15 at a dose of 25 mg/kg and 50 mg/kg significantly ($P < 0.05$) decreased the number and/or severity of neurologic symptoms in animals after pathology modeling. A more pronounced effect was observed when the compound was administered at a dose of 50 mg/kg.

The behavioral status of the rats was assessed using the test "elevated cross-shaped labyrinth" [Table 2]. In the group with a four vascular 4-min cerebral ischemia, a

**Table 1: Effect of LHT 4–15 on the dynamics of the severity of neurologic disorders in rats with cerebral ischemia by McGraw in the modification of I.V. Gannushkina (1996) (based on the average score in the group) (M±m; *n*=10)**

| Groups | Time | | | |
|---|---|---|---|---|
| | 1 day | 3 day | 7 day | 14 day |
| Intact | 0 | 0 | 0 | 0 |
| Pseudo-operated | 0 | 0 | 0 | 0 |
| Pathology | 3.95±0.57 | 2.75±0.29 | 2.4±0.27 | 2.3±0.21 |
| LHT 4–15 25 mg/kg | 1.8±0.11* | 1.75±0.14* | 1.3±0.08* | 1.1±0.1 9* |
| LHT 4–15 50 mg/kg | 0.7±0.09* | 0.6±0.12* | 0.3±0.10* | 0.3±0.10* |

Here and elsewhere * - $P<0.05$ in relation to the control

**Table 2: Effect of LHT 4–15 on the behavioral activity of animals with cerebral ischemia in the test "elevated cross-shaped labyrinth" (M±m; *n*=10)**

| Criterion | Groups | | | | |
|---|---|---|---|---|---|
| | Intact | False-operated | Control | LHT 4-15 25 mg/kg | LHT 4-15 50 mg/kg |
| Dark sleeve, t | 86.8±12.3 | 120±7.1 | 167.3±2.9 | 118±10.7* | 129±7.3* |
| Light sleeve, t | 93.2±12.3 | 60±7.1 | 12.7±2.9 | 64.7±11.8* | 51.4±7.3* |
| Racks, pieces | 5.8±0.6 | 4.4±0.7 | 2.9±0.5 | 5.1±0.5* | 4.9±0.9* |
| Sewing, pieces | 6.9±0.7 | 4.9±0.3 | 1±0.2 | 4.5±0.4* | 3±0.5* |

**Table 3: Effect of LHT 4–15 on the behavioral activity of animals with cerebral ischemia in the actinometry test (M±m; *n*=10)**

| Criterion | Groups | | | | |
|---|---|---|---|---|---|
| | Intact | Pseudo-operated | Pathology | LHT 4–15 25 mg/kg | LHT 4–15 50 mg/kg |
| General activity, y.e. | 775±52.02 | 688.8±29.87 | 451.9±45.05* | 598.9±27.37* | 565.1±45.60* |
| Stereotypes of motion, y.e. | 73.1±2.71 | 55.2±4.56 | 27.4±3.00* | 33.2±2.51* | 38.4±3.52* |
| Maximum speed, y.e. | 34.57±1.82 | 30.29±2.17 | 20.88±1.68* | 23.08±1.40* | 26.68±1.51* |
| Total distance, y.e. | 1497.14±36.67 | 1386.8±51.25 | 756.30±72.11* | 983.78±51.92* | 1055.92±124.76* |
| Time of rest, y.e. | 75.53±13.23 | 98.15±6.02 | 162.17±17.17* | 136.88±10.14* | 129.7±13.52* |

**Table 4: The concentration of markers of brain damage in the plasma of animals at 3 days (M±m; *n*=10)**

| Intact | | Pseudo-operated | | Pathology | | LHT 4–15 25 mg/kg | | LHT 4–15 50 mg/kg | |
|---|---|---|---|---|---|---|---|---|---|
| S100b | NSE | S100b | NSE | S100b | NSE | S100b | NSE | S100b | NSE |
| 0.69±0.11 | 0.415±0.16 | 0.87±0.16 | 0.494±0.13 | 1.91±0.16* | 0.561±0.19* | 0.612±0.14* | 0.367±0.12* | 0.545±0.02# | 0.239±0.11# |

NSE: Neuron-specific enolase

significant decrease in horizontal activity was observed in animals, an increase in the time spent in the dark sleeves. Reduction of vertical activity was manifested in the reduction of the posts, hanging by approximately 75%. The minimal orienting research behavior remained. In the behavioral test "elevated cross-shaped labyrinth" of a group of rats with pathology and preliminary administration of LHT 4–15 at a dose of 25 mg/kg and 50 mg/kg, they showed themselves more actively than the control group. This manifested itself in an increase in horizontal and vertical activity, which was reflected in more racks and stilts. The estimated research behavior declined, but insignificantly ($P < 0.05$). A group of rats with the administration of LHT 04–15 at a dose of 25 mg/kg exhibited an insignificantly higher activity compared to the group administered with 50 mg/kg.

When assessing the motor activity of animals in the actinometry test with IR monitoring of the IR Actimeter activity after the pathology modeling, the activity of the rats dropped: General activity decreased, the number of stereotyped motions, the maximum speed, and the total distance. The rest time, in comparison with the false-operated ones, increased [Table 3].

Groups of animals with the preliminary administration of LHT 4–15 at doses of 25 and 50 mg/kg were more active, developed a high speed, and passed a longer distance. The rest time in these groups is lower in comparison with the control ($P < 0.05$) [Table 3].

Then, the level of s100b and NSE neuron-specific markers in these groups was monitored. In the analysis of S100b and NSE levels in animals of these experimental groups, an increase in the concentration of markers in serum was recorded. A statistically insignificant ($P > 0.05$) increase in the concentration of markers was observed in falsely operated animals. Statistically significant ($P < 0.05$) was an increase in the concentration in the group with 4-min and cerebral ischemia. When analyzing the level of S100b and NSE in animals of the group with cerebral ischemia, with a preliminary introduction of LHT 4–15 at a dose of 50 mg/kg, there was a decrease in the levels of markers in Virginia even below the level of the control group ($P > 0.05$). [Table 4].

Thus, the results indicate a pronounced correction of ischemic brain damage in the conditions of total four-vascular model of cerebral ischemia in rats LHT 04–15 at a dose of 50 mg/kg of body weight of the animal with a single intragastric administration.

## CONCLUSION

Thus, the data obtained from the neurological deficit and in the behavioral tests of the experimental groups confirm the theory of the presence of ethyl thiadiazole derivatives under the LHT code 4–15 neuroprotective

properties. In the group of rats with the administration of the substance in a dose of 50 mg/kg, the effect is more pronounced. In a group of rats with the management of LHT 4–15 at a dose of 25 mg/kg, the neuroprotective effect was also observed, but less pronounced. In this regard, it can be assumed that LHT 4–15 has antioxidant and antihypoxic activity and blocks activation of free radical processes, as well as lipid peroxidation of cell membranes that occur during the development of acute myocardial infarction, ischemic and hemorrhagic strokes, acute violations of regional, and general blood circulation. The level of neuron-specific markers confirms it.

## REFERENCES


1. Spasov AA, Fedorchuk VY, Gurova NA, Reznikov EV. Methodological approach for the study of neuroprotective activity in the experiment. Bull Sci Cent Expertise Med Appl 2014;4:39-45.
2. Shabelnikova A. Correction of ischemic damage to the retina on application of pharmacological preconditioning of recombinant erythropoietin. Res Results Pharmacol 2016;2:67-90.
3. Gureev VV, Alehin SA, Pokrovskiy MV, Dolghikov AA, Korokin MV, Gudyrev OS, *et al*. Remote ischemic preconditioning correction in ADMA-like gestosis model. Res J Pharm Biol Chem Sci 2014;5:1095-8.
4. Stepenko YV, Soldatov VO, Zatolokina MA, Mayorova AV, Sysuev BB, Demidenko AN, *et al*. Stimulation of reparation in a linear wound model in rats by bischofit gel. Pharm Pharmacol 2019;7:42-52.
5. Schenone S, Bruno O, Ranise A, Bondavalli F, Filippelli W, Falcone G, *et al.* 3-arylsulphonyl-5-arylamino-1,3,4-thiadiazol-2(3H)ones as anti-inflammatory and analgesic agents. Bioorg Med Chem 2001;9:2149-53.
6. Martynova O. Tadalafil as an agent of pharmacological preconditioning in ischemic reperfusion brain injury. Res Results Pharmacol 2017;3:20-36.
7. Juszczak M, Walczak K, Langner E, Karpińska M, Matysiak J, Rzeski W, *et al*. Neuroprotective activity of 2-amino-1,3,4-thiadiazole derivative 4BrABT-an *in vitro* study. Ann Agric Environ Med 2013;20:575-9.
8. Kravchenko D, Avdeeva N, Korokin M. Assessment of the DNA damage level in peripheral blood leukocytes of mice treated orally with rapitalam in acute and therapeutic doses. Res Results Pharmacol 2016;2: 9-11.
9. Korokin MV, Pokrovsky MV, Novikov OO, Gureev VV, Denisyuk TA, Korokina LV, *et al.* Effect of L-arginine, Vitamin B6 and folic acid on parameters of endothelial dysfunction and microcirculation in the placenta in modeling of L-NAME-induced NO deficiency. Bull Exp Biol Med 2011;152:70-2.
10. Peresypkina A, Pazhinsky A, Pokrovskii M, Beskhmelnitsyna E, Pobeda A, Korokin M, *et al.* Correction of experimental retinal ischemia by l-isomer of ethylmethylhydroxypyridine malate. Antioxidants (Basel) 2019;8:E34.
11. Soldatova VA, Demidenko AN, Soldatov VO, Deikin AV, Bushueva OY, Puchenkova OA. Hypoxia-inducible factor: Basic biology and involvement in cardiovascular pathology. Asian J Pharm 2018;12:1173-8.
12. Ivlitskaya I, Korokin M, Loktionov A. Pharmacological efficiency of statins and L-norvalin at an endotoxin-induced endothelial dysfunction. Res Results Pharmacol 2016;2:25-35.
13. Pokrovskii MV, Korokin MV, Kudryavtsev KV, Pokrovskaya TG, Gudyrev OS, Gureev VV, *et al.* Study of endothelial protective activity of phenol-derived thrombin and arginase-2 inhibitors KUD-259 and KUD-974. Bull Exp Biol Med 2017;163:436-8.
14. Korokina LV, Zhernakova NI, Korokin MV, Pokopejko ON. Principles of pharmacological correction of pulmonary arterial hypertension. Res Results Pharmacol 2018;4:59-76.
15. Margaritis M, Channon KM, Antoniades C. Statins as regulators of redox state in the vascular endothelium: beyond lipid lowering. Antioxid Redox Signal 2014;20:1198-215.
16. Korokin MV, Pokrovskii MV, Kochkarov VI, Gudyrev OS, Korokina LV, Pokrovskaya TG, *et al*. Endothelial and cardio protective effects of tetrahydrobiopterin, L-norvaline, L-arginine and their combinations by simulation of hyperhomocysteine induced endothelial dysfunction. Res J Pharm Biol Chem Sci 2014;5:1375-9.
17. Ragulina V, Kostina D, Dovgan A, Burda Y, Nadezhdin S. Nuclear factor kappa b as a potential target for pharmacological correction endothelium-associated pathology. Res Results Pharmacol 2017;3:114-24.
18. Kalmykov V, Kusov P, Yablonskaia M, Korshunov E, Korshunova D, Kubekina M, *et al*. New personalized genetic mouse model of Lesch-Nyhan syndrome for pharmacology and gene therapy. Res Results Pharmacol 2018;4:97-104.
19. Abdel-Wahab B. Synthesis and antimicrobial evaluation of some 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and 1,2,4-triazolo[3,4-b] [1,3,4]-thiadiazine derivatives including a 5-(benzofuran-2-yl)-1-phenylpyrazole moiety. Ahmed Monatsh Chem 2009;140:601-5.
20. Almajan GL, Innocenti A, Puccetti L, Manole G, Barbuceanu S, Saramet I, *et al.* Carbonic anhydrase inhibitors. Inhibition of the cytosolic and tumor-associated carbonic anhydrase isozymes I, II, and IX with a series of 1,3,4-thiadiazole- and 1,2,4-triazole-thiols. Bioorg Med Chem Lett 2005;15:2347-52.
21. Amir M, Kumar H, Javed S. Condensed bridgehead nitrogen heterocyclic system: synthesis and pharmacological activities of 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives of ibuprofen and biphenyl-4-yloxy acetic acid. Eur J Med Chem 2008;43:2056-66.
22. Camoutsis C, Geronikaki A, Ciric A, Soković M, Zoumpoulakis P, Zervou M, *et al.* Sulfonamide-1,2,4-thiadiazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity, and conformational studies. Chem Pharm Bull (Tokyo) 2010;58:160-7.


Source of support: Nil; Conflict of interest: None Declared